

## REVIEW

# When versatility matters: activins/inhibins as key regulators of immunity

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Activins and inhibins are members of the transforming growth factor- $\beta$  superfamily that have been considered crucial regulators of cell processes, such as differentiation, proliferation and apoptosis, in different cell types. Initial studies about the function of activin A in the immune system focused on the regulation of hematopoiesis in the bone marrow under homeostatic and inflammatory conditions. Recent data provide a more comprehensive understanding about the role of activins/inhibins in the immune system. Novel findings included in this review point out the important requirement of activin/inhibin signaling to maintain the balance between homeostatic and inflammatory signals that are required for the optimal development and function of immune cells. The purpose of this review is to highlight the versatile nature of activins/inhibins as key regulators of both the innate and adaptive immune responses.

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Activins and inhibins are members of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily that were initially described as regulators of reproductive processes in mammals, and were first named after their positive and negative effect in the release of follicle-stimulating hormone from the pituitary gland, respectively.<sup>1</sup> These dimeric ligands have been shown to modulate a variety of cellular functions such as apoptosis, proliferation, differentiation, cellular migration, among others.<sup>2,3</sup>

Activin A ( $\beta\text{A}/\beta\text{A}$ ) is the best-characterized ligand of this subfamily, although there are other bioactive forms, activin B ( $\beta\text{B}/\beta\text{B}$ ) and activin AB ( $\beta\text{A}/\beta\text{B}$ ), which differ in their potency and cellular function.<sup>4</sup> Activin A is a molecule with pleiotropic functions, which shares with TGF- $\beta$  the SMAD2/3 canonical signaling pathway (reviewed by Shi and Massagué<sup>5</sup>) but uses distinct type II (ActRIIs) and type I (ALK2, ALK4 and ALK7) receptors.<sup>6–9</sup> The biological function of activins can be blocked by follistatin (FS), a glycoprotein that binds activin A with high affinity, functioning as a ligand trap.<sup>10</sup>

On the other hand, inhibins are heterodimers formed by uncommon  $\alpha$ - and  $\beta$ -subunits and exist in two isoforms, inhibin A ( $\alpha/\beta\text{A}$ ) and B ( $\alpha/\beta\text{B}$ ). Inhibins have been shown to antagonize activin-mediated functions by a mechanism that involves binding to  $\beta$ -glycan (TGFBR1II) and formation of a ternary complex with ActRIIs. As a consequence, activin type I receptors (ALK4 and ALK2) are excluded from the receptor complex, leading to the inhibition of SMAD-mediated signaling.<sup>3,11–14</sup> Despite the fact that inhibins have been classically considered as activin antagonists, there is evidence showing that they do not always antagonize activin-mediated functions in

several cell types, suggesting the existence of an independent inhibin-mediated signaling pathway.<sup>3</sup>

Compelling evidence has shown that activins also regulate various non-reproductive processes, such as mesoderm induction, liver proliferation, skin morphogenesis, erythropoiesis, bone formation, neuron survival and immune function.<sup>15–17</sup>

Most of the immunological studies have focused on the role of activin A, because of the fact that it shares a common signaling pathway with TGF- $\beta$ . Indeed, activin A has emerged as a genuine cytokine that can be considered a key regulator of the immune system in mammals. Interestingly, many studies have shown that activin A displays pro- and anti-inflammatory properties depending on both cellular and temporal contexts. In fact, activin A has been involved in different clinical inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, allergic airway inflammation and clinical sepsis condition.<sup>18–22</sup> This review examines the recent findings about the role of activin signaling on immune cells, including the crosstalk with the Toll-like receptor (TLR) pathway, the impact in hematopoiesis and lymphocyte development, the effects on dendritic cell (DC) maturation, its emergence as a novel T helper cell type 2 (Th2) cytokine and its recent role in the induction of regulatory T-cell subsets. Collectively, these data support the role of activins/inhibins as key orchestrators of the immune system in homeostasis and inflammation.

## ROLE OF ACTIVINS/INHIBINS IN INNATE IMMUNE CELLS

A great amount of evidence has accumulated supporting the role of activins and inhibins in the regulation of cell function. Table 1

**Table 1** Effects of activins/inhibins in immune cell types

(a)	Cell type	Experimental system	Effects	Reference
Monocytes	Human			
	<i>In vitro</i>	Monocytes (PBMCs)	TNF- $\alpha$ , LPS, IL-1 $\alpha$ , dihydroxyvitamin D3, IFN- $\gamma$ , GM-CSF, CD40-CD40L stimulation $\uparrow$ activin A expression	24,25,26,29
		Monocytes (PBMCs), THP-1 and U-937 cells	Glucocorticoids and retinoic acid $\downarrow$ activin A expression Activin A $\uparrow$ IL-6, IL-1 $\alpha$ and TNF- $\alpha$ , (resting), but $\downarrow$ IL-1 $\beta$ and $\uparrow$ IL-1ra in activated monocytes	30,31
Macrophages	Mouse			
	<i>In vitro</i>			
		Peritoneal macrophages	TLR2 (Pam3cys), -4 (LPS) and -9 (CpG) $\uparrow$ activin A secretion	35
		Bone marrow-derived macrophages, peritoneal macrophages and cell line RAW264.7	Activin A $\uparrow$ NO, IL-1 $\beta$ , PGE $_2$ , TXA $_2$ , TNF- $\alpha$ and IL-6, but in activated macrophages $\downarrow$ NO and IL-1 $\beta$	36,37,39 <sup>a</sup> , 41,47,49
		Peritoneal macrophages and RAW264.7 cell line	Activin A $\uparrow$ phagocytosis and pinocytosis, but $\downarrow$ these activities under activation conditions	37,41,47
		Peritoneal macrophages and RAW264.7 cell line	Activin A $\uparrow$ MHC-II and CD80 expression, but $\downarrow$ MHC-II, CD68, CD14 and TLR4 induced by LPS	37,41,47
		Peritoneal macrophages	Activin A $\uparrow$ MMP-2 production	38
		Bone marrow-derived macrophages	Activin A promotes osteoclastogenesis	45,46
	<i>In vivo</i>			
		<i>In vivo</i> phagocytosis assay	Activin A $\downarrow$ phagocytosis induced by LPS	41
Microglial cells	Human			
	<i>In vitro</i>	THP-1 cells differentiated to macrophage phenotype	Activin A $\downarrow$ TNF- $\alpha$ and IL-8 induced by LPS	44
Microglial cells	Mouse			
	<i>In vitro</i>			
		Microglial cells	TLR2 (Pam3cys), -4 (LPS) and -9 (CpG) $\uparrow$ activin A	35,50
		MG6 microglial cell line	Activin A $\downarrow$ IL-18, IL-6 and iNOS induced by LPS	50
Mast cells	Mouse			
	<i>In vitro</i>			
		RBL-2H3 mast cell line and bone marrow-derived mast cell progenitors	IgE receptor crosslinking $\uparrow$ activin A	56,57 <sup>a</sup>
		Bone marrow-derived mast cell progenitors	Activin A regulates cell growth and maturation of mast cells	58,59
		Bone marrow-derived mast cell progenitors	Activin A $\uparrow$ cell migration and production of MCP-1, -6 and -7	59,60,61
	<i>In vivo</i>			
		OVA challenge of mast cell-deficient mice (WBB6F1-W/W <sup>-</sup> )	OVA challenge $\uparrow$ activin A in mast cells	57
	Human			
	<i>In vitro</i>			
		Mast cells	IgE receptor crosslinking $\uparrow$ activin A	57
NK cells	<i>In vivo</i>			
		Lung tissues from normal and asthmatic patients	Activin A expression restricted to mast cells	57
NK cells	Human			
	<i>In vitro</i>	Human NK cells alone or co-cultures with mo-DCs	DC-derived activin A $\downarrow$ NK cell proliferation $\downarrow$ IFN- $\gamma$ , IL-6, TNF- $\alpha$ , GM-CSF, IL-1 $\beta$ , MIP-1 $\beta$ , MIP-1 $\alpha$ , IL-8, IP-10 $\uparrow$ IL-10 production	73
(b) DCs	Mouse			
	<i>In vitro</i>			
		Skin explant cultures and mo-DCs	Activin A $\uparrow$ LC migration	71,74
	<i>In vivo</i>			
		Epidermal sheets from transgenic mice overexpressing follistatin	$\downarrow$ LC numbers in the epidermis	71
DCs	Human			
	<i>In vitro</i>			
		LC derived from CD14 <sup>+</sup> monocytes	Activin A promoted LC differentiation	70
		Intradermal injection of activin A in human skin explants cultures		

Table 1 (Continued)

Cell type	Experimental system	Effects	Reference
	Mo-DCs and peripheral CD11c <sup>+</sup> myeloid DCs	CD40L and agonists for TLR4 (LPS), -3 (poly-I:C) and -7/8 (R848) ↑ activin A	72
	Co-cultures of mo-DCs and autologous T cells	FS blocking of CD40L-induced activin A ↑ IL-10, TNF- $\alpha$ , IL-6 and IL-12p70, MCP-1, RANTES, IL-8 and IP-10 and ↑ T-cell proliferation	
	Immature mo-DCs and peripheral DCs	Activin A ↑ CXCL12, CXCL14, MMP2, MMP3 and MMP9 production	74
	Mo-DCs cultures	Activin A and inhibin A ↓ maturation of mo-DC	75
B cells	Mouse		
	<i>In vitro</i>		
	Splenic B cells	LPS ↑ activin A, ↓ FS and activin receptors	86
	Long-term bone marrow cultures	↓ Functional activin A T-B-cell lymphopoiesis	84,85
	B9 and MPC-11 cell lines	Activin A promotes apoptosis	82
	Splenic B cells and CH12F3-2 B-cell lymphoma	Activin A sensitizes resting B cells to produce IgG after LPS activation	86,88
		Activin A ↑ IgA production induced by LPS	
	<i>In vivo</i>		
	Lymphoid tissues (B6/c mice)	LPS challenge ↓ activin A expression in bone marrow and spleen	84,85
	Intrabone marrow and intraspleen injections of actA-MEFs	Activin A delays B-cell development (↑ pre-pro B cells and ↓ late pro-pre B cells)	
	OVA-immunized Balb/c mice	OVA-stimulated splenic B cells ↑ activin A, ↓ FS and activin receptors	86
		Activin A ↑ IgE secretion through other immune cells	
	Human		
	<i>In vitro</i>		
	Purified lymphocytes	Activin A induces apoptosis	87,88
T cells	Mouse		
	<i>In vitro</i>		
	Rat thymocytes and peripheral blood T lymphocytes	Activin A ↓ thymocyte and mature T-cell growth and inhibin ↑ thymocyte proliferation	97-100 <sup>a</sup>
	Fetal and adult thymus	Activin receptor and Smads is preferentially expressed DN subset	101,102
		Inhibins are the major ligand expressed in thymic stroma	
		Activin A triggered more Smad2 phosphorylation on immature thymocytes	
	FOTCs derived from wild-type and inhibin $\alpha$ -null mice	Activin A and inhibin A regulate DN3-DN4, DN-DR, DP-CD8SP transitions	103
		Activin $\alpha$ -/- thymus show ↓ cellularity, ↑ %DN and ↓ %DP thymocytes	
	Th2 skewed cells	TCR crosslinking (CD3 or OVA) of Th2 cells ↑ activin A production	49
	OVA-specific Th2 clones		
	Suppression of OVA-specific T cells by activin A-treated CD4 <sup>+</sup> T cells	Activin A- induced CD4 <sup>+</sup> regulatory T cells (Foxp3 <sup>+</sup> ) suppress Th2 responses through actions of IL-10 and TGF- $\beta$	63
	Naive T lymphocytes	Activin A promotes and synergizes with TGF- $\beta$ in the conversion of naive T cells into Foxp3 <sup>+</sup> iTregs	104
	<i>In vivo</i>		
	OVA-induced allergic airway inflammation model	Endogenous activin A blocking ↓ regulatory CD4 <sup>+</sup> T-cell subset (Foxp3 <sup>+</sup> ) and protection of mice from allergic airway disease	63
	K14-activin BA transgenic mice		
	K14-follistatin transgenic mice	Activin A ↑ the conversion of naive T cells into Foxp3 <sup>+</sup> iTreg	104

Abbreviations: DC, dendritic cell; DN, double negative; DP, double positive; Foxp3, forkhead box P3; FS, follistatin; FOTC, fetal thymic organ culture; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN- $\gamma$ , interferon- $\gamma$ ; Ig, immunoglobulin; IL, interleukin; NOS, inducible nitric oxide synthase; iTreg, adaptive Foxp3<sup>+</sup>CD4<sup>+</sup> regulatory T cell; Langerhans cells; LPS, lipopolysaccharide; MCP-1, macrophage chemoattractant protein 1; MEF, mouse embryonic fibroblast; MHC-II, major histocompatibility complex class II; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; mo-DC, monocyte-derived DC; NK, natural killer; NO, nitric oxide; OVA, ovalbumin; PBMC, peripheral blood mononuclear cell; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; Tbx21, Thymocyte 21.

The table lists the activins/inhibins effects in immune cell types emphasizing the experimental strategies and the species employed.

<sup>a</sup>Experimental data obtained from rat species.

summarizes the effects of these ligands in different immune cell types, both in the mouse and human systems.

## Monocytes

Initial studies investigated the role of activin A in the regulation of hematopoiesis in the bone marrow under homeostatic and inflammatory conditions (reviewed by Shav-Tal and Zipori<sup>23</sup>). Activin A, expressed by monocytes and bone marrow stromal cells, was shown to promote growth and differentiation of multipotent progenitor cells and erythroid precursor cells.<sup>24-27</sup> In response to inflammatory

signals, such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), lipopolysaccharide (LPS), interleukin-1  $\alpha$  (IL-1 $\alpha$ ), bone marrow monocytes and stromal cells secrete activin A,<sup>24,28</sup> whereas other stimuli, such as glucocorticoids and retinoic acid, downregulate activin A expression in granulocyte-macrophage colony-stimulating factor (GM-CSF)-derived monocytes.<sup>29</sup> In addition, an alternative mechanism for the induction of activin A by human bone marrow monocytes is through the cognate interaction with activated T cells requiring the CD40-CD40L interaction and T-cell-derived cytokines such as GM-CSF and interferon- $\gamma$  (IFN- $\gamma$ ).<sup>29</sup>

On the other hand, GM-CSF, LPS and phorbol myristate acetate stimulation can also induce activin A secretion by peripheral blood monocytes.<sup>25,26</sup> It has been proposed that activin A stimulates IL-6 production by human peripheral blood monocytes and indirectly promotes the synthesis of immunoglobulin (Ig)E by B cells.<sup>30</sup> However, activin A may also act as an anti-inflammatory factor regulating monocyte activation through the inhibition of interleukin-1 $\beta$  production and concomitant increase of IL-1 receptor antagonist expression.<sup>31</sup>

# Macrophages

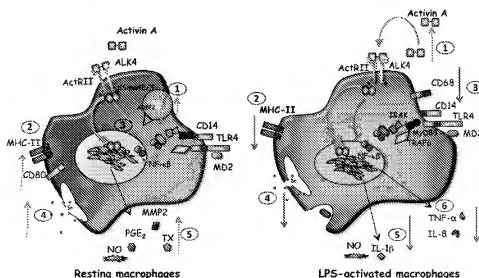
Macrophages, which are central effector cells of the innate immunity, have been proposed to be a cell source of activin A during the induction of a systemic inflammatory response (reviewed by Phillips et al.<sup>16</sup>). *In vivo* experiments based on LPS challenge in different animal models have established that activin A clearly shows a biphasic pattern of release into the circulation, preceding the release of its inhibitor FS. In this context, treatment with exogenous FS has confirmed the important requirement of the early release of activin A to regulate IL-1 $\beta$ , TNF- $\alpha$  and IL-6 secretion induced by LPS.<sup>22,32-34</sup> Moreover, recent evidence has shown that macrophages are able to secrete activin A in response to the activation of TLR2, TLR4 and TLR9 (Figure 1 (1), right panel).<sup>35-39</sup> Accordingly, elevated activin A levels have been observed in a mouse model of endotoxemia and in patients with clinical sepsis condition.<sup>22,40</sup> Furthermore, treatment with FS increased the survival of mice that were lethally challenged with LPS,<sup>22</sup> supporting the role of activin A as an early proinflammatory mediator in these models.

On the other hand, many reports have shown the versatile nature of activin A in regulating macrophage functions, functioning as a proinflammatory cytokine in steady-state macrophages or showing anti-inflammatory actions under activation conditions (Figure 1). For

example, activin A can induce the synthesis of prostanoids, such as prostaglandin E2 and thromboxane A<sub>2</sub>, nitric oxide (NO) and IL-1 $\beta$  in bone marrow macrophages,<sup>39</sup> but in the presence of LPS signals, activin A downregulates inducible nitric oxide synthase mRNA, NO and IL-1 $\beta$  release by macrophages (Figure 1 (5), left and right panels).<sup>36,37,41</sup>

A mechanism proposed for the induction of NO release, induced by activin A in resting macrophages, involves the induction of a positive-feedback signal, which upregulates ActRIIA on the cell surface, through the expression of ARIP2 (activin-interacting receptor protein; Figure 1 (1), left panel).<sup>36</sup> Similarly, in LPS-activated macrophages, ActRIIA expression is also required for NO downregulation induced by activin A.<sup>36</sup> Paradoxically, stimulation of mouse peritoneal macrophages by LPS was shown to downregulate the mRNA of type II (ActRIIA and ActRIIB) receptors, whereas the expression of ActRIIB (ALK4) was not altered.<sup>38</sup>

An alternative mechanism proposed for the negative effect of activin A on LPS-mediated responses is based on the downregulation of TLR4-mediated signal transduction through the decrease of surface CD14 and CD68 expression (Figure 1 (2), right panel).<sup>37,41</sup> In fact, recent evidence shows the existence of a crosstalk between TLR and activin signal-transduction pathways, which, in the case between TGF- $\beta$  and TLR signaling, has been well documented.<sup>42,43</sup> Thus, activin A decreases the phosphorylation of extracellular signal-regulated kinase 1/2, p38, Jun N-terminal kinase, mitogen-activated protein kinases and the p65 subunit of NF- $\kappa$ B (nuclear factor kappa-light-chain enhancer of activated B cells), which are classical effectors of the TLR4/CD14/MD2 signaling in LPS-stimulated macrophages, leading to a reduced TNF- $\alpha$  and IL-8 production (Figure 1 (6), right panel).<sup>44</sup> Moreover, this effect was dependent on the maintenance of SH2-containing inositol 5'-phosphatase levels, which were enhanced by



**Figure 1** Activin A regulates macrophage function depending on their activation state. Left panel, in steady-state conditions, activins can exert a proinflammatory effect in macrophages. (1) Activin A establishes a positive-feedback signal increasing ActRIIA on the cell surface through the expression of ARIP2, (2) upregulates the expression of MHC class II and costimulatory molecules, (3) induces nuclear translocation of NF- $\kappa$ B, (4) augments phagocytic activity and (5) promotes the secretion of cytokines, inflammatory mediators and matrix metalloproteinase-2. Right panel, on activation, (1) activin A secretion is augmented and may act in an autocrine manner inducing a (2) decrease in antigen presentation, through the downregulation of MHC class II expression. In addition, (3) activin A diminishes TLR4-mediated signaling through the downregulation of CD14 and CD68 surface expression, (4) downregulates the phagocytic and pinocytotic activities of activated macrophages, (5) affects the production of NO and IL-1 $\beta$  and (6) attenuates NF- $\kappa$ B activity induced by LPS, resulting in a reduced TNF- $\alpha$  and IL-8 production. IRAK, IL-1R-associated kinase; MMP-2, matrix metalloproteinase-2; MyD88, myeloid differentiation primary response gene 88; PGE<sub>2</sub>, prostaglandin E2; TRAF6, TNF receptor-associated factor 6; TXA<sub>2</sub>, thromboxane A<sub>2</sub>.

insulin. These results are in accordance with the positive effects of insulin treatment in the control of septic shock.<sup>44</sup> In contrast, activin A by itself has the capacity to stimulate I- $\kappa$ B activation, nuclear translocation of NF- $\kappa$ B (Figure 1 (3), left panel) and phosphorylation of ERK1/2 and p38 mitogen-activated protein kinase in murine bone marrow macrophages under basal conditions or after stimulation with receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) and M-CSF.<sup>45,46</sup>

On the other hand, activin A can impair macrophage functions by downregulating the expression of major histocompatibility complex (MHC) class II molecule and pinocytotic/phagocytic activities in LPS-treated macrophages, thus reducing their antigen-presenting function (Figure 1 (2) and (4), right panel).<sup>37,41,47</sup> Conversely, activin A upregulates MHC class II and CD80 expression, as well as phagocytic and pinocytotic activities in resting macrophages (Figure 1 (2) and (4), left panel).<sup>47</sup>

In addition, activin A also may regulate migration and tissue infiltration of activated macrophages during an immune response. The release of matrix metalloproteinases is known to promote cell migration and is especially relevant during cancer cell invasion (reviewed by Kessenbrock *et al.*<sup>48</sup>). Activin A induces the secretion of type IV collagenase matrix metalloproteinase-2 in resting and activated mouse peritoneal macrophages.<sup>38</sup> In this way, activin A may potentially act as a regulator of macrophage migration.

Finally, recent evidence clearly indicates that activin A can function as a Th2 cytokine promoting the alternative activation of macrophages (M2 phenotype). It was shown that activin A secreted by differentiated CD4<sup>+</sup> Th2 cells is able to induce the expression of arginase-1 and, in parallel, decrease the expression of IFN- $\gamma$ -induced inducible nitric oxide synthase.<sup>49</sup>

Altogether, the aforementioned studies highlight the role of activin A as a crucial component of macrophage function under homeostatic and inflammatory conditions, regulating activation, release of inflammatory mediators and, possibly, cell migration and antigen presentation. However, more studies are necessary to address the *in vivo* contribution of activin signals in macrophage-mediated functions.

### Microglial cells

Activin A has been shown to have an anti-inflammatory role on microglial cells, which are the major immunocompetent cell type in the central nervous system. As described for conventional macrophages, TLR2, TLR4 and TLR9 signaling also increases activin A levels in microglial cells.<sup>35,50</sup> In addition, activin A modulates microglial activation induced by LPS, reducing the production of IL-6, IL-18 and inducible nitric oxide synthase. However, in contrast to what was described for conventional macrophages, activin A also acts as anti-inflammatory factor in microglial cells under steady-state conditions.<sup>50</sup>

Increased levels of activin A are found in the brain of rabbits with meningitis, and its expression appears to be restricted locally to activated microglial cells and infiltrating macrophages.<sup>51</sup> In addition, patients with meningitis condition showed elevated levels of activin A and FS in cerebrospinal fluid, although this was not found in a mouse model of meningitis.<sup>52,53</sup> In this context, it has been suggested that FS has a role in controlling activin A-mediated functions during infection and inflammation of the central nervous system.<sup>53</sup> On the other hand, activin A released in CFS, in conditions of traumatic brain injury, has also been postulated to act as a neuroprotective factor in central nervous system.<sup>54</sup>

### Mast cells

Mast cells are innate cells that are capable of eliciting rapid responses through the release of several proinflammatory mediators, which in

turn regulate immune cell trafficking and the early establishment of an inflammatory process. These cells have been extensively studied because of their role in mediating type I hypersensitivity reactions. Thus, mast cells are activated through the aggregation of high-affinity Fc $\epsilon$ R1 receptors induced by antigen-IgE complexes, through activation of TLRs, or by complement components.<sup>55</sup> Once mast cells are activated, they degranulate within seconds to minutes, releasing a large array of pre-packaged mediators into the surrounding tissue, including proteases, histamine, heparin and TNF- $\alpha$ . Furthermore, mast cells release a second wave of *de novo*-synthesized cytokines such as IL-4, IL-6, IL-3, IL-5 and TNF- $\alpha$ , as well as eicosinoids such as leukotrienes.<sup>55</sup>

Human and murine mast cells secrete activin A after IgE receptor (Fc $\epsilon$ R1) crosslinking or after phorbol myristate acetate stimulation, within hours and increase over time. Similar to other cytokines, activin A appears to be secreted in a second wave of mediators by mast cells, thus requiring *de novo* synthesis at a transcriptional level.<sup>56,57</sup> Moreover, activin A is only observed in the cytoplasm of IgE-activated bone marrow-derived murine mast cells, whereas in resting state this cytokine is not detected.<sup>57</sup>

Mast cell degranulation is a process that is highly dependent on the induction of cytosolic Ca<sup>2+</sup>. Similarly, the release of activin A is also dependent on the activation of the Ca<sup>2+</sup>-calmodulin-dependent enzymes, calcineurin and CamK, which synergize with p38 mitogen-activated protein kinase and c-Jun NH<sub>2</sub>-terminal kinase activity to further regulate activin  $\beta$ A subunit transcription.<sup>56</sup>

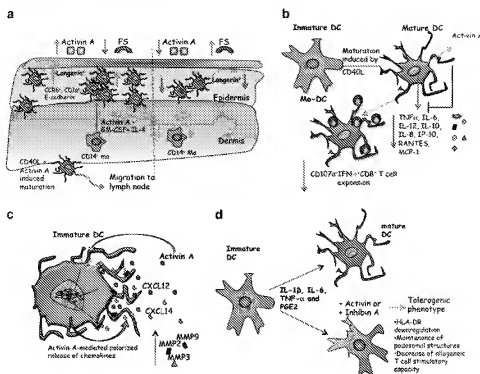
Activin A, similar to TGF- $\beta$ , has the potential to modulate differentiation, growth and migration of mast cells. Activin A stimulated the differentiation of mast cells, which are characterized by a rounded and compact cell morphology and by the presence of metachromatic granules.<sup>58</sup> In this study, activin A was also shown to inhibit cell growth of a mast cell line in response to IL-3. However, a later study has reported that activin A and TGF- $\beta$  do not always act as growth inhibitors, but rather may function as survival factors.<sup>59</sup> Moreover, activin A induces mast cell migration and promotes the expression of murine mast cell protease-1 (MCP-1), a chymase related with the maturation of mucosal mast cells.<sup>58</sup> Other studies also reported that tryptases MCP-6 (ref. 60) and MCP-7 (ref. 61) are upregulated by activin A, although their possible effect on mast cell migration was not evaluated.

Compelling data indicate that mast cells are a cell source of activin A during allergic airway inflammation and in asthma condition.<sup>57</sup> In this context, high levels of serum activin A have been found in patients with severe asthma and in bronchoalveolar lavage fluid from mice challenged with ovalbumin (OVA),<sup>62,63</sup> which correlated with increased p-Smad2 levels in bronchial biopsies.<sup>21</sup> Recent data have shown that activin A production can also be induced by IL-13, a cytokine involved in mucus production, which is a characteristic feature of asthma pathogenesis.<sup>64</sup> Interestingly, recent data indicate that activin A can have a protective role during allergic airway inflammation, suppressing Th2 responses through the generation of antigen-specific regulatory T cells.<sup>63</sup>

### Dendritic cells

DCs are specialized cells that have an important role in adaptive immunity, inducing tolerance or antigen-specific immunity. Irrespective of their ontogeny, from myeloid or lymphoid precursor cells, there are many reports showing the influence of activin/inhibin signaling on Langerhans cells (LCs) and conventional DCs.

The function of LC is required to regulate inflammatory and repair processes in the epidermis of skin and mucosa. Both processes are



**Figure 2** Activin A modulates LC differentiation and DC function. (a) Activin A can induce the differentiation of LC from CD14<sup>+</sup> monocytes, accumulating Langerin (CD207)<sup>+</sup> cells in the epidermis. During the maturation of LC, activin A can synergize with CD40L to increase the surface expression of CD80, CD86, CCR7 and CXCR4, leading to enhanced LC migration and T-cell stimulation. Conversely, FSH overexpression leads to a substantial reduction in the number of Langerin<sup>+</sup> cells in the epidermis due to a reduced LC migration. (b) Activin A, released by mo-DC during CD40L-mediated maturation, acts in an autocrine manner to diminish functional properties of DCs, such as the induction of proinflammatory mediators and antigen-specific CD8<sup>+</sup> T cell expansion. (c) Activin A induces DC migration through the polarized release of CXCL12 and CXCL14. In addition, activin A promotes the secretion of activated forms of metalloproteinase-2, -3 and -9. (d) Activin A and inhibin A negatively regulate human DC maturation induced by proinflammatory signals, inducing a tolerogenic phenotype. CCL5 (RANTES), chemokine (C-C motif) ligand 5; CCR6, CC chemokine receptor 6; CD1a, cluster of differentiation 1a; CXCL12, chemokine (C-X-C motif) ligand 12; 14, HLA-DR, human leukocyte antigen DR; IP-10 (CXCL10), interferon-inducible protein-10; MCP-1 (CCL2), chemokine (C-C motif) ligand 2; mo-DC, monocyte-derived DC

regulated by activin signals, which becomes evident from the phenotype of  $\beta A$ -deficient mice, showing a clear deficiency in whisker development.<sup>65</sup> In accordance with these results, transgenic overexpression of activin A under the K14 promoter, which restricts expression to non-differentiated basal cells of the epidermis and keratinocytes of the outer root sheath of the hair follicles, causes an enhanced wound healing and altered skin morphogenesis.<sup>66,67</sup> Interestingly, when these mice were crossed with transgenic mice expressing a dominant-negative ActRIB under the same promoter, a slight delay of wound repair and partial rescue of skin abnormalities were observed, which suggests that activin affects both stromal cells and keratinocytes. In addition, after injury, mice overexpressing FSH show a delayed wound repair and scar formation.<sup>68</sup>

Activin A has been shown to regulate LC activation, differentiation and migration. Under inflammatory conditions, LCs become activated and migrate to the regional lymph node, increasing, in parallel, surface MHC class II, CD80 and CD86 and acquiring the capacity to present antigens to naive T lymphocytes.<sup>69</sup> In this context, activin A possesses a function similar to that of TGF- $\beta$  in promoting the differentiation of LC from CD14<sup>+</sup> monocytes (Figure 2a). In the presence of GM-CSF and IL-4, activin A promotes the expression of LC markers *in vitro*, similar to that promoted by TGF- $\beta$ , including Langerin (CD207), CD1a, E-cadherin, CLA and CCR6 (Figure 2a). In addition, both activin A and TGF- $\beta$  downregulate the expression of CD14 and, in

parallel, upregulate activin A mRNA, thus establishing an autocrine loop of newly synthesized activin A that enhances LC differentiation.<sup>70</sup> A similar effect was observed after intradermal injection of activin A in human skin explants.<sup>70</sup> Conversely, mice overexpressing FSH show reduced LC numbers in the epidermis compared with the wild-type mice, supporting the positive role of activin signaling in LC differentiation (Figure 2a).<sup>71</sup> In addition, decreased numbers of LCs were obtained in supernatants from whole ear skin and epidermal sheet cultures of these mice. Although FSH was shown to regulate the trafficking of LCs, no differences in the expression of maturation markers were observed in these mice.<sup>71</sup> Finally, activin A is capable of generating functionally competent LCs, after CD40 ligation, which is characterized by the upregulation of surface CD80, CD83, CXCR4 and CCR7 (Figure 2a), and production of high levels of IL-12, TNF- $\alpha$ , CCL20 and CCL22, leading to the induction of allogeneic T-cell responses.<sup>70</sup>

Activin A can also be induced in other DC subtypes under inflammatory conditions. For example, LPS or CD40L stimulation was shown to induce a rapid activin A release in monocyte-derived DCs and peripheral blood CD1c<sup>+</sup> DCs, but not in plasmacytoid DCs.<sup>72</sup> In addition, other TLR agonists, such as R-848 (TLR7/8), Pam3Cys (TLR2) and poly I:C (TLR3), can also induce the production of activin A by monocyte-derived DCs.<sup>72</sup>

Recently, new evidence has shown that activin A has the potential to negatively regulate proinflammatory responses and mediate peripheral

tolerance through the regulation of T-cell stimulatory capacity of different DC subsets. Activin A released after CD40L activation, but not after LPS stimulation, appears to downregulate cytokine and chemokine production by monocyte-derived DCs in an autocrine/paracrine manner. This effect is evidenced by the inhibition of CD40L-induced activin A with FS, which results in an enhanced cytokine (IL-10, TNF- $\alpha$ , IL-6 and IL-12p70) and chemokine (MCP-1, RANTES, IL-8 and IP-10) production (Figure 2b).<sup>72</sup> Furthermore, the inhibition of activin A signaling by FS resulted in an enhanced frequency of CD107a<sup>+</sup>, IFN- $\gamma$ <sup>+</sup> CD8<sup>+</sup> effector T cells (Figure 2b).<sup>72</sup>

In addition, recent data have shown that activin A produced by DCs can act as a suppressor of natural killer functions, negatively regulating natural killer cell proliferation, IFN- $\gamma$  production and cytokine and chemokine expression, although it does not affect their cytotoxic activity.<sup>73</sup> In this regard, activin A may serve as a key regulator not only of DC function but also of innate cells, such as natural killers, specialized in the recognition and attack of transformed and virus-infected cells.

As mentioned previously, CD40L-induced activin A regulates DC migration by modulating chemokine and chemokine receptor expression.<sup>70,72</sup> However, a recent report has shown *ex vivo* that activin A can function as a chemotactic factor in an indirect manner, inducing a polarized release of CXCL12 and CXCL14 from immature monocyte-derived DCs and circulating myeloid DCs (Figure 2c).<sup>74</sup> Moreover, activin A has the ability to upregulate the production of matrix-metalloproteinase-2, -3 and -9 in migrating DCs to further degrade extracellular matrix components (Figure 2c).<sup>74</sup>

Recent evidence indicates that inhibins can also modulate DC functions. In this context, inhibin A, considered a tumor suppressor factor, may have a tolerogenic role preventing DC maturation in the human pregnancy decidua.<sup>75</sup> It is worth noting that inhibin  $\alpha$ -deficient mice display a cachexia-like syndrome, which is accompanied by an inflammatory process, which may be in part explained by the appearance of tumors in these mice.<sup>76–80</sup> Furthermore, activin A and inhibin A were able to counteract the expression of human leukocyte antigen-DR induced after maturation of human DCs by IL-1 $\beta$ , IL-6, TNF- $\alpha$  and prostaglandin E<sub>2</sub>, although they had no influence on CD40, CD83 and CD86 expression (Figure 2d).<sup>75</sup> In addition, both ligands affected the generation of the classic morphology of mature DCs and impaired the induction of T-cell proliferation, similar to that observed from DCs treated with TGF- $\beta$  or dexamethasone (Figure 2d).<sup>75</sup>

In summary, activin/inhibin signaling regulates DC functions in steady-state and inflammatory conditions through several mechanisms, although their contribution in the modulation of the immune response against pathogens and tumor cells still remains unexplained.

## ROLE OF ACTIVINS/INHIBINS ON ADAPTIVE IMMUNE CELLS B cells

Activin A, initially designated as Restrictin P, is considered a key regulator of B-cell development in the bone marrow, owing to its ability to control the processes of cell cycle arrest and apoptosis (reviewed by Shav-Tal and Zipori<sup>23</sup> and Zipori and Barda-Saad<sup>81</sup>). These effects are in part related to the classical antagonism described for activin A on IL-6 signals, inhibiting B-cell proliferation and promoting death cell.<sup>81</sup> In fact, activin A itself, similar to TGF- $\beta$ , has the ability to induce apoptosis in B lymphoid cells through Smad-dependent expression of the lipid phosphatase SH2-containing inositol 5'-phosphatase as a mechanism to at least counteract the survival signals that are AKT-mediated by IL-6.<sup>82</sup>

Evidence has pointed out the importance of activin A and its soluble inhibitor FS in the regulation of B-cell lymphopoiesis under steady-state and inflammatory conditions. Recent data have confirmed the inverse relationship between the stromal expression of activin A and the occurrence of B-cell development (Figure 3a). In this context, IL-1 $\beta$  upregulates activin A and, in parallel, diminishes FS secretion, potentially affecting B-cell differentiation, whereas IFN- $\gamma$  signals act inversely by suppressing activin A and augmenting FS expression by human bone marrow stromal fibroblasts (Figure 3a).<sup>83</sup> Moreover, *in vivo* polyclonal B-cell activation induced by LPS injection was accompanied by a notable reduction of  $\beta$ A expression in bone marrow and spleen (Figure 3a).<sup>84</sup>

Several experiments have shown that the inhibitory signals provided by activin A occur at early stages of B-cell development. Analysis of B-cell lymphopoiesis *in vitro* showed that functional activin A is observed at early stage of cultures, whereas the appearance of B220<sup>+</sup> B-cell colonies correlated with a lower tier of functional activin A and high levels of FS and inhibin  $\alpha$ -chain expression (Figure 3a).<sup>84</sup> In addition, using mouse embryonic fibroblast cell line that over-expresses activin A (actA-MEFs) as a stromal cell support, the frequency of B220<sup>+</sup> cells was reduced and the injection of actA-MEFs resulted in an accumulation of immature B lineage cells *in vivo*.<sup>85</sup> Conversely, the use of a mouse endothelialidocyte cell line, which lacks functional activin A, resulted in the early accumulation of CD43<sup>+</sup>B220<sup>+</sup> and CD43<sup>+</sup>B220<sup>+</sup> B-cell colonies.<sup>84</sup>

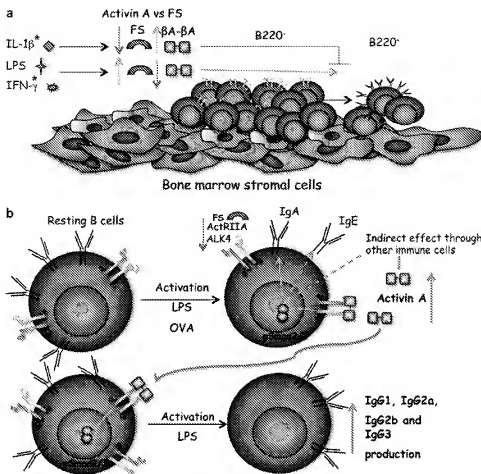
Hence, activin A behaves as a "brake" signal on B-cell differentiation under steady conditions, but once inflammatory signals have emerged, such as those triggered by LPS, activin A expression in the bone marrow is turned off, allowing B-cell differentiation and the expansion of the B-cell repertoire (Figure 3a).

On the other hand, several studies indicate that activin A is able to shape B-cell function depending on its activation state (Figure 3b). In resting splenic B cells, activin  $\beta$ A subunit and FS mRNA are moderately expressed, whereas the activin  $\beta$ B mRNA is absent.<sup>86</sup> However, after LPS stimulation, B cells secrete high levels of activin A and concomitantly diminish the secretion of its inhibitor FS, as well type II (ActRIIA and ActRIIB) and type I (ActRIIB) receptors,<sup>86</sup> similar to that previously mentioned for macrophages.<sup>38</sup> This inverse relationship between activin A and the expression of FS and activin receptors was also observed in B cells isolated from OVA-immunized mice, an established model to evaluate a Th2-type immune responses. In this model, neutralization of activin A induced significant reduction of serum IgE levels, indicating that activin A has an important role during Th2 responses.<sup>86</sup> However, activin A is not able to directly induce IgE production by B cells, but rather may influence the activation of other immune cells (Figure 3b).<sup>30,35,58</sup>

In addition, *in vitro* studies suggest that activin A acts on resting B cells, but not on activated B cells to stimulate IgG production. Preincubation of B cells with activin A and subsequent stimulation with LPS promote an enhanced B-cell proliferation and upregulation with IgG1, IgG2a, IgG2b and IgG3 (Figure 3b).<sup>86</sup>

Finally, recent data suggest that activin A has the ability to regulate mucosal immunity through the regulation of IgA isotype expression. More specifically, activin A enhanced IgA expression induced by LPS, and this effect appeared to be mediated by the induction of GLT<sub>2</sub> (Ig germline  $\alpha$ ) and PST<sub>2</sub> (post-switch transcripts  $\alpha$ ) in B-lymphoma cells and normal mouse-spleen B cells (Figure 3b).<sup>87,88</sup>

Thus, activin A released by activated B cells has been proposed to regulate the antibody isotype switching in the spleen, acting on resting B cells, which, once activated, show upregulation of IgG isotype antibodies and, in this way, participate in the fine tuning of humoral



**Figure 3** Activin A regulates B-cell development and function. **(a)** An inverse correlation exists between stromal expression of functional activin A and B-cell differentiation in the bone marrow. IL-1 $\beta$  can induce activin A and suppress FS production, as a possible mechanism to delay B-cell differentiation, leading to the accumulation of B220 $^{+}$  precursor cells. Conversely, LPS and IFN- $\gamma$  upregulate FS and suppress activin A expression, thereby promoting B-cell development. **(b)** Activin A regulates B-cell immunoglobulin production depending on its activation state. Activin A can directly induce IgA production through increasing Ig germline  $\alpha$  (GLT $\alpha$ ) and post-switch  $\alpha$  transcripts (PST $\alpha$ ). In addition, activin A can induce IgE production possibly with the participation of other immune cells, such as macrophages and mast cells. Activated B cells secrete considerable levels of activin A, which may induce resting B cells to produce IgG after an LPS challenge. Asterisks denote proposed mechanisms.

responses. So far, it is unknown whether the expression and signaling of activin A can be regulated directly by the B-cell receptor as a possible crosstalk between both pathways.

Finally, activin A may be involved in the regulation of inflammatory diseases in which IL-6 is overexpressed, such as rheumatoid arthritis<sup>89</sup> (reviewed by Gribi et al.<sup>19</sup>). In this context, high levels of activin A were found in synovial fluid of rheumatoid arthritis patients, potentially released by CD68 $^{+}$  macrophage lineage cells, synoviocytes and chondrocytes in response to IL-1 $\beta$ , IFN- $\gamma$ , TGF- $\beta$  and IL-8. Activin A was shown to downregulate several IL-6-mediated functions, including B-cell proliferation, fibrinogen production and phagocytic activity of monocytes.<sup>90</sup> However, activin A has also been found to promote cell proliferation of synovial fibroblasts,<sup>91</sup> indicating that more studies are still necessary to establish the pathophysiological role of activin A in rheumatoid arthritis.

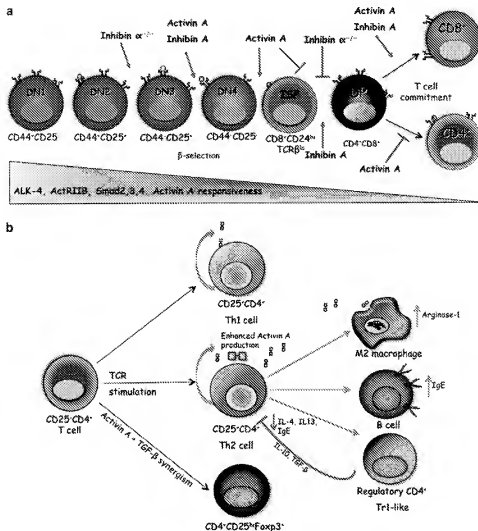
#### T cells

TGF- $\beta$  is considered a critical regulator of T-cell-mediated immunity, and has been shown to regulate T-cell development, peripheral

homeostasis and tolerance to self-antigens (reviewed by Li and Flavell<sup>92</sup>). However, the role of activins in T-cell immunobiology has been less explored. As these members share a conserved signaling pathway, it is not surprising that they affect similar functions in T cells.

As it is well known, the development of a self-restricted and auto-tolerant T-cell repertoire takes places in the thymus, through a highly regulated process that involves the differentiation, migration and selection of developing thymocytes (reviewed by Takahama<sup>93</sup>). During thymocyte maturation, four major subsets can be identified on the basis of the expression of surface CD4 and CD8 co-receptors: double negative (DN, CD4 $^{-}$ CD8 $^{-}$ ), double positive (DP, CD4 $^{+}$ CD8 $^{+}$ ) and single positive (CD4 $^{+}$  or CD8 $^{+}$ ). The most immature subset (DN) can be further subdivided into four subpopulations on the basis of the expression of CD44 and CD25: DN1 (CD44 $^{+}$ CD25 $^{-}$ ), DN2 (CD44 $^{+}$ CD25 $^{+}$ ), DN3 (CD44 $^{+}$ CD25 $^{+}$ ) and DN4 (CD44 $^{+}$ CD25 $^{-}$ ) (Figure 4a).

T-cell development requires T-cell receptor (TCR)-mediated interactions with self-peptide-MHC complexes expressed on thymic stromal cells. Thus, TCR signaling sets the avidity/affinity threshold that



**Figure 4** Activins and inhibins regulate T-cell differentiation. (a) ALK4, ActRIIB and Smads 2, 3 and 4 are expressed in fetal and adult thymi, preferentially in early developmental stages. Activins and inhibins regulate the DN to DP transition, as well as DP to CD4<sup>+</sup> and CD8<sup>+</sup>SP transition, in FTOC. In addition, analysis of FTOCs derived from inhibin  $\alpha$ -deficient mice show delayed thymocyte development, specifically at the transitions DN2-DN3 and DN-DP. (b) Activin A regulates the induction of peripheral regulatory T cells and promotes Th2 responses. TCR and CD28 signaling induces activin A and TGF- $\beta$  during the conversion of naive CD25<sup>+</sup>CD4<sup>+</sup> T cells to effector CD25<sup>+</sup>CD4<sup>+</sup> T cells. TGF- $\beta$  and activin A secreted from other cell types synergize in the conversion of iTregs (CD25<sup>+</sup>Foxp3<sup>+</sup>CD4<sup>+</sup>T cells), leading to an enhanced suppression of activated T cells in the periphery. In addition, activin A secretion is augmented under Th2-skewing conditions. Th2 activin A-producing cells induce the differentiation of M2 macrophages through the upregulation of arginase-1. On the other hand, activin A, produced during a Th2 inflammatory response, such as asthma, has a protective role in inducing the generation of antigen-specific regulatory T cells, which secrete IL-10 and TGF- $\beta$  (Tr1-like). Foxp3, forkhead box P3; IL-10, interleukin 10; iTreg, inducible Foxp3<sup>+</sup>CD4<sup>+</sup> regulatory; TCR- $\beta$ , T-cell receptor beta.

dictates thymocyte fate, leading to cell death by negligence or negative selection, or rescue from apoptosis by positive selection (reviewed by Starr *et al.*<sup>94</sup>). In addition to cell-to-cell interactions, soluble molecules released by stromal cells, such as chemokines, cytokines and growth factors, are key factors regulating the process of thymocyte differentiation (reviewed by Petrie and Zuniga-Pflucker<sup>95</sup>). Among the soluble mediators controlling the cellular processes involved in thymocyte development, the members of TGF- $\beta$  superfamily have been shown to have an important role (reviewed by Licona-Limon and Soldevila<sup>96</sup>). In this regard, members of activin/inhibin subfamily are not an exception. Initial observations about the role of activin/inhibin signaling in T cells were described *in vitro*. Activin A and TGF- $\beta$  can suppress the proliferation of rat thymocytes and peripheral

lymphocytes induced by suboptimal doses of phorbol myristate acetate and concanavalin A, whereas inhibin antagonizes these effects, promoting thymocyte proliferation.<sup>97–99</sup> Similar to the effect described on B cells, activin A reduces T-cell proliferation through the down-regulation of endogenous IL-6 production.<sup>100</sup>

Our group and others have recently reported the expression of signaling components of the activin/inhibin pathways in the murine thymus.<sup>101,102</sup> Specifically, BA,  $\beta$ B and  $\alpha$  ligand subunits are expressed mainly in stromal cells, although inhibins appeared to be the most highly expressed ligand both in fetal and adult thymus.<sup>101</sup> On the other hand, expression of activin receptors was more pronounced in the DN immature subpopulation, indicating that this subset is more susceptible to activin signaling (Figure 4a).<sup>101</sup> In accordance with the

expression pattern of activin receptors in the DN subset, activin A phosphorylated Smad2 mostly in immature thymocytes (CD4<sup>+</sup> CD25<sup>-</sup>, CD4<sup>+</sup> CD25<sup>+</sup>), whereas TGF- $\beta$  induced p-Smad2 mainly in mature CD4<sup>+</sup> and CD8<sup>+</sup> SP thymocytes.<sup>102</sup>

Moreover, we have recently shown that activin/inhibin signals regulate specific checkpoints during T-cell development. Despite the functional antagonism reported in reproductive cells, both activin A and inhibin A added in fetal thymic organ cultures promoted DN3 to DN4 transition, suggesting their positive role during the  $\beta$ -selection process (Figure 4a).<sup>103</sup> Nevertheless, these factors appeared to differentially regulate DN4–DP transition. Activin A induced an accumulation of an intermediate single-positive subpopulation (CD8<sup>+</sup> HSA<sup>hi</sup> TCR<sup>lo</sup>), whereas inhibin A, instead, promoted the transition from DN4 to DP (Figure 4a).<sup>103</sup> On the other hand, both ligands can promote CD8<sup>+</sup> SP cell differentiation, suggesting their potential role in CD8<sup>+</sup> T-cell commitment (Figure 4a).<sup>103</sup> These data indicate that inhibins do not antagonize activin-mediated functions during thymocyte development, as described above for DC maturation.<sup>79</sup> Finally, functional analysis of inhibin  $\alpha$ -deficient mice at fetal stages showed a marked reduction in total cell numbers and a reduced DN–DP transition, suggesting its potential role as a regulator of crucial cellular processes in thymocytes, including proliferation, cell cycle progression and apoptosis (Figure 4a).<sup>103</sup>

Recent evidence has shown the versatile nature of activin A in regulating T-cell responses, depending on the cytokine microenvironment under which T-cell activation occurs.

Activin A has emerged as a novel Th2 cytokine that can be released by activated CD4<sup>+</sup> T cells. When CD4<sup>+</sup> T cells are differentiated into Th cell subsets, higher activin secretion is detected when cultured under Th2-skewing conditions (Figure 4b).<sup>49</sup> Similarly, production of activin A is enhanced in OVA-specific Th2 clones in response to OVA, or after anti-CD3 crosslinking.<sup>49</sup> Indeed, the basic region leucine/zipper transcription factor c-Maf, which is induced during Th2 differentiation, appears to synergize with NFAT to promote transcriptional activation of the activin  $\beta$ A gene.<sup>49</sup>

As mentioned above, activin A was shown to promote the alternative activation of macrophages (M2) and indirectly the production of IgE by naive B cells, which are characteristic features of a Th2-mediated response (Figure 4b).<sup>49</sup> However, recent data have evidenced that activin A also has a protective role in suppressing antigen-specific and Th2 responses through the generation of antigen-specific regulatory T cells. Hence, blocking of endogenous activin A *in vivo* during OVA-induced allergic responses results in an enhanced airway hyper-responsiveness, leukocyte infiltration and release of inflammatory mediators.<sup>63</sup> The regulatory CD4<sup>+</sup> T-cell subset induced by activin A was able to suppress antigen-specific Th2 cell proliferation and Th2-mediated responses. This suppressive action of activin A-induced regulatory CD4<sup>+</sup> T cells was mediated by IL-10 and TGF- $\beta$ , and as these cells were devoid of Foxp3 expression, they possibly represent a Tr1-like phenotype (Figure 4b).<sup>63</sup> Finally, *in vivo* transfer of activin-A-induced regulatory CD4<sup>+</sup> T cells, or systemic administration of recombinant activin A, resulted in protection from allergic airway inflammation, with a concomitant reduction in leukocyte infiltration, mucus production and airway hyper-responsiveness.<sup>63</sup>

On the other hand, activin A has also been involved in the generation of Foxp3<sup>+</sup>-induced regulatory T cells. Indeed, activin A can induce, *in vitro*, the conversion CD25<sup>-</sup>CD4<sup>+</sup> naive T cells into CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells after CD3 plus CD28 stimulation, although to a lesser extent compared with TGF- $\beta$  (Figure 4b).<sup>104</sup> In addition, activin A was shown to synergize with TGF- $\beta$  in the generation of Foxp3<sup>+</sup>-induced regulatory T cells, which were capable

of suppressing the proliferation of CD25<sup>-</sup>CD4<sup>+</sup>-naive T cells (Figure 4b).<sup>104</sup> These studies also showed that TGF- $\beta$ , but not activin A, released in an autocrine manner, was essential during the conversion of naive T cells into functional Tregs.<sup>104</sup> In addition, *in vivo* experiments using transgenic mice that overexpress activin A under the control of the human K14 promoter, showed an increment in peripheral, but not central, CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> T cells.<sup>102</sup>

Altogether, these studies highlight the function of activins/inhibins in T-cell biology, by influencing T-cell development and effector T-cell responses. As previously mentioned for other immune cells, activins also act as pleiotropic factors in T cells, depending on their differentiation state, the cytokine milieu and the context under which T-cell activation occurs.

## Concluding remarks

Although very early data established a role for activin and inhibin in the regulation of immune cell function, in the recent years, a plethora of evidence points out their versatility in the control of immune responses. As described, activins/inhibins can function as autocrine/paracrine factors that, under the influence of the cytokine microenvironment, contribute to establish a complex regulatory network that shapes the innate and adaptive immune response.

In this review, we have described that activins regulate the synthesis of inflammatory cytokines, cell growth, cell migration and apoptosis in different immune cells. However, many questions remain unresolved, such as the specific contribution of activins/inhibins in the development of autoimmune diseases, their role in the differentiation of specific Th cell lineages (that is, Th1, Th17, Th9), or in the maturation of peripheral B-cell subsets (T1, T2, MZB, and so on). In addition, as activin A has been shown to modulate DC function and promote in the induction of regulatory T cells, we could hypothesize that they may have a role in anti-tumour immunity. Finally, inhibins do not always antagonize activin-mediated functions; therefore, it is feasible to propose that they may exert differential effect on immune cells, although this has not yet been explored.

In conclusion, activins/inhibins regulate the interphase between tolerance and immunity; however, more studies are required to elucidate whether activin/inhibin signaling components could serve as potential targets for immune intervention.

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